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FILE 'REGISTRY' ENTERED AT 14:32:05 ON 28 APR 2004

=> S GLUCORONYL C5 EPIMERASE/CN
L1             0 GLUCORONYL C5 EPIMERASE/CN

=> S GLUCURONIC ACID EPIMERASE/CN
L2             0 GLUCURONIC ACID EPIMERASE/CN

=> S GLUCURONATE EPIMERASE/CN
L3             0 GLUCURONATE EPIMERASE/CN

=> S GLUCURONYL(3W)EPIMERASE;S GLUCURONIC ACID EPIMERASE;S GLUCURONATE EPIMERASE
            1147 GLUCURONYL
               1 GLUCURONYLS
            1147 GLUCURONYL
                   (GLUCURONYL OR GLUCURONYLS)
            2188 EPIMERASE
             260 EPIMERASES
            2229 EPIMERASE
                   (EPIMERASE OR EPIMERASES)
L4            15 GLUCURONYL(3W)EPIMERASE


           11551 GLUCURONIC
         3810244 ACID
         1430347 ACIDS
         4276490 ACID
                   (ACID OR ACIDS)
            2188 EPIMERASE
             260 EPIMERASES
            2229 EPIMERASE
                   (EPIMERASE OR EPIMERASES)
L5             5 GLUCURONIC ACID EPIMERASE
                   (GLUCURONIC(W)ACID(W)EPIMERASE)


            2101 GLUCURONATE
             131 GLUCURONATES
            2188 GLUCURONATE
                   (GLUCURONATE OR GLUCURONATES)
            2188 EPIMERASE
             260 EPIMERASES
            2229 EPIMERASE
                   (EPIMERASE OR EPIMERASES)
L6            11 GLUCURONATE EPIMERASE
                   (GLUCURONATE(W)EPIMERASE)

=> S L4,L5,L6
L7            29 (L4 OR L5 OR L6)

=> S MOUSE
          296845 MOUSE
              27 MOUSES
          365258 MICE
              28 MICES
L8        551075 MOUSE
                   (MOUSE OR MOUSES OR MICE OR MICES)

=> S MURINE
           98758 MURINE
```

21 MURINES
L9 98775 MURINE
(MURINE OR MURINES)

=> S (L8,L9) AND L7
L10 4 ((L8 OR L9)) AND L7

=> S L7 NOT L10
L11 25 L7 NOT L10

=> D L10 1-4 CBIB ABS;D L11 1-25 CBIB ABS

L10 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN
2003:579443 Document No. 139:211372 Targeted Disruption of a **Murine Glucuronyl** C5-**epimerase** Gene Results in Heparan Sulfate Lacking L-Iduronic Acid and in Neonatal Lethality. Li, Jin-Ping; Gong, Feng; Hagner-McWhirter, Asa; Forsberg, Erik; Abrink, Magnus; Kisilevsky, Robert; Zhang, Xiao; Lindahl, Ulf (Department of Medical Biochemistry and Microbiology, Biomedical Center, University of Uppsala, Uppsala, SE-751 23, Swed.). Journal of Biological Chemistry, 278(31), 28363-28366 (English) 2003. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB The glycosaminoglycan, heparan sulfate (HS), binds proteins to modulate signaling events in embryogenesis. All identified protein-binding HS epitopes contain L-iduronic acid (IdoA). We report that targeted disruption of the **murine** D-**glucuronyl** C5- **epimerase** gene results in a structurally altered HS lacking IdoA. The corresponding phenotype is lethal, with renal agenesis, lung defects, and skeletal malformations. Unexpectedly, major organ systems, including the brain, liver, gastrointestinal tract; skin, and heart, appeared normal. We find that IdoA units are essential for normal kidney, lung, and skeletal development, albeit with different requirement for 2-O-sulfation. By contrast, major early developmental events known to critically depend on heparan sulfate apparently proceed normally even in the absence of IdoA.

L10 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN
2002:449840 Document No. 137:29828 Cloning and characterization of **mouse glucuronyl** C5-**epimerase**, and use of N-terminal fragments of the epimerase in fusion protein constructs. Jalkanen, Markku; El Darwish, Kamel; Lindahl, Ulf; Li, Jin-Ping (Biotie Therapies Corp., Finland). PCT Int. Appl. WO 2002046379 A2 20020613, 58 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-FI1068 20011207. PRIORITY: US 2000-PV304180 20001208.

AB The invention is directed to a novel purified **mouse glucuronyl** C5-**epimerase**, fragments thereof, nucleic acids encoding the same and the recombinant production thereof. A **mouse** liver gene encoding **glucuronyl** C5- **epimerase** was cloned. The cDNA sequence and the encoded amino acid sequence of the **mouse** liver C5-epimerase are provided. The amino acid sequence of the C5-epimerase was found to be 618 amino acids long, with a mol. weight of 71.18 kDa. The invention is also directed to fragments of such epimerase, especially N-terminal fragments that are useful in fusion protein constructs to enhance the activity of recombinantly- produced heterologous epimerase enzymes.

L10 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN
2001:436414 Document No. 135:163947 Characterization of the D-**glucuronyl** C5-**epimerase** involved in the biosynthesis of heparin and heparan sulfate. Li, Jin-Ping; Gong, Feng; El Darwish, Kamel; Jalkanen, Markku; Lindahl, Ulf (Department of Medical Biochemistry and Microbiology, Biomedical Center, University of Uppsala, Uppsala, SE-751 23, Swed.). Journal of Biological Chemistry, 276(23), 20069-20077 (English) 2001. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB The **murine** gene for the **glucuronyl** C5- **epimerase** involved in heparan sulfate biosynthesis was cloned, using a previously isolated bovine lung cDNA fragment as probe. The .apprx.11-kilobase pair **mouse** gene contains 3 exons from the first ATG to stop codon and is localized to chromosome 9. Southern anal. of the genomic DNA and chromosome mapping suggested the occurrence of a single epimerase gene. Based on the genomic sequence, a **mouse** liver cDNA was isolated that encodes a 618-amino acid residue protein, thus extending by 174 N-terminal residues the sequence deduced from the (incomplete) bovine cDNA. Comparison of **murine**, bovine, and human epimerase cDNA structures indicated 96-99% identity at the amino acid level. A cDNA identical to the **mouse** liver species was demonstrated in **mouse** mast cells committed to heparin biosynthesis. These findings suggest that the iduronic acid residues in heparin and heparan sulfate, despite different structural contexts, are generated by the same C5-epimerase enzyme. The catalytic activity of the recombinant full-length **mouse** liver epimerase, expressed in insect cells, was found to be > 2 orders of magnitude higher than that of the previously cloned, smaller bovine recombinant protein. The .apprx.52-kDa, similarly highly active, enzyme originally purified from bovine liver was found to be associated with an .apprx.22-kDa peptide generated by a single proteolytic cleavage of the full-sized protein.

L10 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN
1997:729676 Document No. 128:58849 Biosynthesis of heparin/heparan sulfate. cDNA cloning and expression of D-**glucuronyl** C5-**epimerase** from bovine lung. Li, Jin-Ping; Hagner-Mcwhirter, Asa; Kjellen, Lena; Palgi, Jaan; Jalkanen, Markku; Lindahl, Ulf (Department of Medical and Physiological Chemistry, University of Uppsala, Uppsala, S-751 23, Swed.). Journal of Biological Chemistry, 272(44), 28158-28163 (English) 1997. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB **Glucuronyl** C5-**epimerases** catalyze the conversion of D-glucuronic acid (GlcUA) to L-iduronic acid (IdceA) units during the biosynthesis of glycosaminoglycans. An epimerase implicated in the generation of heparin/heparan sulfate was previously purified to homogeneity from bovine liver (Campbell, P., Hannesson, H. H., Sandback, D., Roden, L., Lindahl, U., and Li, J.-p. (1994) J. Biol. Chemical 269, 26953-26958). The present report describes the mol. cloning and functional expression of the lung enzyme. The cloned enzyme contains 444 amino acid residues and has a mol. mass of 49,905 Da. N-terminal sequence anal. of the isolated liver enzyme showed this species to be a truncated form lacking a 73-residue N-terminal domain of the deduced amino acid sequence. The coding cDNA insert was cloned into a baculovirus expression vector and expressed in Sf9 insect cells. Cells infected with recombinant epimerase showed a 20-30-fold increase in enzyme activity, measured as release of 3H2O from a polysaccharide substrate containing C5-3H-labeled hexuronic acid units. Furthermore, incubation of the expressed protein with the appropriate (GluUA-GlcNSO3)n substrate resulted in conversion of .apprx.20% of the GlcUA units into IdceA residues. Northern anal. implicated two epimerase transcripts in both bovine lung and liver

tissues, a dominant .apprx.9-kilobase (kb) mRNA and a minor .apprx.5-kb species. **Mouse** mastocytoma cells showed only the .apprx.5-kb transcript. A comparison of the cloned epimerase with the enzymes catalyzing an analogous reaction in alginate biosynthesis revealed no apparent amino acid sequence similarity.

L11 ANSWER 1 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN

2004:286251 Irreversible Glucuronyl C5-epimerization in the Biosynthesis of Heparan Sulfate. Hagner-McWhirter, Asa; Li, Jin-Ping; Oscarson, Stefan; Lindahl, Ulf (Arrhenius Laboratory, Sweden and the Department of Organic Chemistry, S-751 23 Uppsala, Box 582, The Biomedical Center, Uppsala University, Department of Medical Biochemistry and Microbiology, Stockholm University, Stockholm, S-106 91, Swed.). Journal of Biological Chemistry, 279(15), 14631-14638 (English) 2004. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB **Glucuronyl** C5-**epimerase** catalyzes the conversion of D-glucuronic acid to L-iduronic acid units in heparan sulfate biosynthesis. Substrate recognition depends on the N-substituent pattern of the heparan sulfate precursor polysaccharide and requires the adjacent glucosamine residue toward the non-reducing end to be N-sulfated. Epimerization of an appropriately N-sulfated substrate is freely reversible in a soluble system, with equilibrium favoring retention of D-gluco configuration (Hagner-McWhirter, Å., Lindahl, U., and Li, J.-P. (2000) BIOCHEM: J. 347, 69-75). We studied the reversibility of the epimerase reaction in a cellular system, by incubating human embryonic kidney 293 cells with D-[5-3H]galactose. The label was incorporated with glucuronic acid units into the heparan sulfate precursor polysaccharide and was lost upon subsequent C5-epimerization to iduronic acid. However, anal. of oligosaccharides obtained by deaminative cleavage of the mature heparan sulfate chains indicated that all glucuronic acid units retained their C5-3H label, irresp. of whether they had occurred in sequences susceptible or resistant to the epimerase. All 3H-labels of the final products resisted incubation with epimerase in a soluble system, apparently due to blocking O-sulfate groups. These results indicate that glucuronic acid C5-epimerization is effectively irreversible in vivo and argue for a stringent organization of the biosynthetic machinery.

L11 ANSWER 2 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN

2004:246172 Document No. 140:232732 Differential sulfations and epimerization define heparan sulfate specificity in nervous system development. Buelow, Hannes E.; Hobert, Oliver (Department of Biochemistry and Molecular Biophysics Center for Neurobiology and Behavior, College of Physicians and Surgeons, Columbia University, New York, NY, 10032, USA). Neuron, 41(5), 723-736 (English) 2004. CODEN: NERNET. ISSN: 0896-6273. Publisher: Cell Press.

AB Heparan sulfate proteoglycans (HSPG) are components of the extracellular matrix through which axons navigate to reach their targets. The heparan sulfate (HS) side chains of HSPGs show complex and differentially regulated patterns of secondary modifications, including sulfations of distinct hydroxyl groups and epimerization of an asym. carbon atom. These modifications endow the HSPG-containing extracellular matrix with the potential to code for an enormous mol. diversity. Attempting to decode this diversity, we analyzed C. elegans animals lacking three HS-modifying enzymes, **glucuronyl** C5-**epimerase**,

heparan 6O-sulfotransferase, and 2O-sulfotransferase. Each of the mutant animals exhibit distinct as well as overlapping axonal and cellular guidance defects in specific neuron classes. We have linked individual HS modifications to two specific guidance systems, the sax-3/Robo and kal-1/Anosmin-1 systems, whose activity is dependent on different HS modifications in different cellular contexts. Our results demonstrate that the mol. diversity in HS encodes information that is crucial for different aspects of neuronal development.

L11 ANSWER 3 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN

2003:1007019 Document No. 140:47541 Epimerized derivatives of K5 polysaccharide with a very high degree of sulfation. Oreste, Pasqua Anna; Zoppetti, Giorgio (Italy). PCT Int. Appl. WO 2003106504 A1 20031224, 65 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-IB2338 20030617. PRIORITY: IT 2002-MI1345 20020618; IT 2002-MI1346 20020618; IT 2002-MI1854 20020827.

AB A method is described for the oversulfation of epiK5-N-sulfate to obtain an epiK5-amine-O-oversulfate with very high sulfation degree which, by subsequent N-sulfation, provides new epiK5-N,O-oversulfate-derivs. with a sulfation degree of at least 4, basically free of activity on the coagulation parameters and useful in the cosmetic or pharmaceutical field. Also described are new low mol. weight epiK5-N-sulfates useful as intermediates in the preparation of the corresponding LMW-epiK5-N,O-oversulfate- derivs.

L11 ANSWER 4 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN

2002:897453 Document No. 138:150106 A Sinorhizobium meliloti lipopolysaccharide mutant altered in cell surface sulfation. Keating, David H.; Willits, Michael G.; Long, Sharon R. (Howard Hughes Medical Institute, Stanford University, Stanford, CA, 94305, USA). Journal of Bacteriology, 184(23), 6681-6689 (English) 2002. CODEN: JOBAAY. ISSN: 0021-9193. Publisher: American Society for Microbiology.

AB The Rhizobium-legume symbiosis involves the formation of a novel plant organ, the nodule, in which intracellular bacteria reduce mol. dinitrogen in exchange for plant photosynthates. Nodule development requires a bacterial signal referred to as Nod factor, which in Sinorhizobium meliloti is a β-(1,4)-linked tetramer of N-acetylglucosamine containing N-acyl and O-acetyl modifications at the nonreducing end and a critical 6-O-sulfate at the reducing end. This sulfate modification requires the action of three gene products: nodH, which catalyzes the sulfonyl transfer, and nodPQ, which produce the activated form of sulfate, 3'-phosphoadenosine-5'-phosphosulfate. It was previously reported that S. meliloti cell surface polysaccharides are also covalently modified by sulfate in a reaction dependent on NodPQ. We have further characterized this unique form of bacterial carbohydrate modification. Our studies have determined that one of the nodPQ mutant strains used in the initial study of sulfation of cell surface harbored a second unlinked mutation. We cloned the gene affected by this mutation (referred to as lps-212) and found it to be an allele of lpsL, a gene previously predicted to encode a UDP- **glucuronic acid epimerase**. We demonstrated that lpsL encoded a UDP-**glucuronic acid epimerase** activity that was reduced in the lps-212 mutant. The lps-212 mutation resulted in an altered lipopolysaccharide structure that was reduced in sulfate modification in vitro and in vivo. Finally, we determined that the

lps-212 mutation resulted in a reduced ability to elicit the formation of plant nodules and by altered infection thread structures that aborted prematurely.

L11 ANSWER 5 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN
2002:888388 Document No. 138:382456 Biosynthesis of Chondroitin/Dermatan Sulfate. Silbert, Jeremiah E.; Sugumaran, Geetha (Dep. Veterans Affairs Med. Cent., Bedford, MA, 01730, USA). IUBMB Life, 54(4), 177-186 (English) 2002. CODEN: IULIF8. ISSN: 1521-6543. Publisher: Taylor & Francis Inc..

AB A review. Chondroitin sulfate and dermatan sulfate are synthesized as galactosaminoglycan polymers containing N-acetylgalactosmine alternating with glucuronic acid. The sugar residues are sulfated to varying degrees and positions depending upon the tissue sources and varying conditions of formation. Epimerization of any of the glucuronic acid residues to iduronic acid at the polymer level constitutes the formation of dermatan sulfate. Chondroitin/dermatan glycosaminoglycans are covalently attached by a common tetrasaccharide sequence to the serine residues of core proteins while they are adherent to the inner surface of endoplasmic reticulum/Golgi vesicles. Addition of the first sugar residue, xylose, to core proteins begins in the endoplasmic reticulum, followed by the addition of two galactose residues by two distinct glycosyl transferases in the early cis/medial regions of the Golgi. The linkage tetrasaccharide is completed in the medial/trans Golgi by the addition of the first glucuronic acid residue, followed by transfer of N-acetylgalactosamine to initiate the formation of a galactosaminoglycan rather than a glucosaminoglycan. This specific N-acetylgalactosaminyl transferase is different from the chondroitin synthase involved in generation of the repeating disaccharide units to form the chondroitin polymer. Sulfation of the chondroitin polymer by specific sulfotransferases occurs as the polymer is being formed. All the enzymes in the pathway for synthesis have been cloned, with the exception of the **glucuronyl** to iduronyl **epimerase** involved in the formation of dermatan residues.

L11 ANSWER 6 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN
2002:506880 Document No. 138:217205 D-**Glucuronyl** C5-**epimerase** in heparin/heparan sulfate biosynthesis. Li, Jin-Ping; Lindahl, Ulf (Department of Medical Biochemistry and Microbiology, Section of Medical Biochemistry, Biomedical Center, Uppsala University, Uppsala, S-751 23, Swed.). Handbook of Glycosyltransferases and Related Genes, 403-409. Editor(s): Taniguchi, Naoyuki; Honke, Koichi; Fukuda, Minoru. Springer-Verlag Tokyo: Tokyo, Japan. ISBN: 4-431-70311-X (English) 2002. CODEN: 69CUXS.

AB A review describes the history, enzyme activity assay and substrate specificity, and biol. aspects of D-**glucuronyl** C5- **epimerase**, a key enzyme in the biosynthesis of heparin and heparan sulfate.

L11 ANSWER 7 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN
2001:634531 Document No. 136:258038 Analysis of the chromosome sequence of the legume symbiont Sinorhizobium meliloti strain 1021. Capela, Delphine; Barloy-Hubler, Frederique; Gouzy, Jerome; Bothe, Gordana; Ampe, Frederic; Batut, Jacques; Boistard, Pierre; Becker, Anke; Boutry, Marc; Cadieu, Edouard; Dreano, Stephane; Gloux, Stephanie; Godrie, Therese; Goffeau, Andre; Kahn, Daniel; Kiss, Erno; Lelaure, Valerie; Masuy, David; Pohl, Thomas; Portetelle, Daniel; Puhler, Alfred; Purnelle, Benedicte; Ramsperger, Ulf; Renard, Clotilde; Thebault, Patricia; Vandenbol, Micheline; Weidner, Stefan; Galibert, Francis (Laboratoire de Biologie Moleculaire des Relations Plantes-Microorganismes, Unite Mixte de

Recherche (UMR) 215 Centre National de la Recherche Scientifique (CNRS), Institut National de la Recherche Agronomique, Chemin, Tolosan, F-31326, Fr.). Proceedings of the National Academy of Sciences of the United States of America, 98(17), 9877-9882 (English) 2001. CODEN: PNASA6. ISSN: 0027-8424. Publisher: National Academy of Sciences.

AB Sinorhizobium meliloti is an α-proteobacterium that forms agronomically important N2-fixing root nodules in legumes. We report here the complete sequence of the largest constituent of its genome, a 62.7% GC-rich 3654,135-bp circular chromosome. Annotation allowed assignment of a function to 59% of the 3341 predicted protein-coding ORFs, the rest exhibiting partial, weak, or no similarity with any known sequence. Unexpectedly, the level of reiteration within this replicon is low, with only two genes duplicated with more than 90% nucleotide sequence identity, transposon elements accounting for 2.2% of the sequence, and a few hundred short repeated palindromic motifs (RIME1, RIME2, and C) widespread over the chromosome. Three regions with a significantly lower GC content are most likely of external origin. Detailed annotation revealed that this replicon contains all housekeeping genes except two essential genes that are located on pSymB. Amino acid/peptide transport and degradation and sugar metabolism appear as two major features of the S. meliloti chromosome. The presence in this replicon of a large number of nucleotide cyclases with a peculiar structure, as well as of genes homologous to virulence determinants of animal and plant pathogens, opens perspectives in the study of this bacterium both as a free-living soil microorganism and as a plant symbiont.

L11 ANSWER 8 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN

2001:628249 Document No. 135:269095 Hexuronyl C5-epimerases in alginate and glycosaminoglycan biosynthesis. Valla, S.; Li, J.-p.; Ertesvag, H.; Barbeyron, T.; Lindahl, U. (Department of Biotechnology, The Norwegian University of Science and Technology, Trondheim, 7491, Norway). Biochimie, 83(8), 819-830 (English) 2001. CODEN: BICMBE. ISSN: 0300-9084. Publisher: Editions Scientifiques et Medicales Elsevier.

AB A review with 55 refs. The sugar residues in most polysaccharides are incorporated as their corresponding monomers during polymerization Here, the authors summarize the 3 known exceptions to this rule, involving the biosynthesis of alginate, and the glycosaminoglycans, heparin/heparan sulfate and dermatan sulfate. Alginate is synthesized by brown seaweeds and certain bacteria, whereas glycosaminoglycans are produced by most animal species. In all cases, one of the incorporated sugar monomers are C5-epimerized at the polymer level, from D-mannuronic acid to L-guluronic acid in alginate, and from D-glucuronic acid to L-iduronic acid in glycosaminoglycans. Alginate epimerization modulates the mech. properties of seaweed tissues, whereas in bacteria it seems to serve a wide range of purposes. The conformational flexibility of iduronic acid units in glycosaminoglycans promotes apposition to, and thus functional interactions with a variety of proteins at cell surfaces and in the extracellular matrix. In Azotobacter vinelandii, the alginates are epimerized at the cell surface or in the extracellular environment by a family of evolutionary strongly related modular type and Ca2+-dependent epimerases (AlgE1-7). Each of these enzymes introduces a specific distribution pattern of guluronic acid residues along the polymer chains, explaining the wide structural variability observed in alginates isolated from nature. Glycosaminoglycans are synthesized in the Golgi system, through a series of reactions that include the C5-epimerization reaction along with extensive sulfation of the polymers. The single, Ca2+-independent, epimerase in heparin/heparan sulfate biosynthesis and the Ca2+-dependent dermatan sulfate epimerase(s) also generate variable epimerization patterns, depending on other polymer-modification reactions. The alginate and heparin epimerases appear unrelated at the amino acid sequence level, and have probably evolved through independent evolutionary pathways; however,

hydrophobic cluster anal. indicates limited similarity. Seaweed alginates are widely used in industry, whereas heparin is well established in the clinic as an anticoagulant.

L11 ANSWER 9 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN
2001:446021 Document No. 135:222925 The **Glucuronyl** C5-**Epimerase** Activity Is the Limiting Factor in the Dermatan Sulfate Biosynthesis. Tiedemann, Kerstin; Larsson, Thomas; Heinegard, Dick; Malmstrom, Anders (Department of Cell and Molecular Biology, Section for Cell and Matrix Biology, University of Lund, Lund, S-22184, Swed.). Archives of Biochemistry and Biophysics, 391(1), 65-71 (English) 2001. CODEN: ABBIA4. ISSN: 0003-9861. Publisher: Academic Press.

AB An early step in the biosynthesis of dermatan sulfate is polymerization to chondroitin, which then is modified by the D-**glucuronyl** C5- **epimerase** and mainly 4-O-sulfotransferase. The final structure of the dermatan sulfate side chains varies and our aim was to identify, which of the two enzymes that are crucial to generate dermatan sulfate copolymeric structures in tissues. Dermatan sulfate side chains of biglycan and decorin were prepared from fibroblasts and nasal and articular chondrocytes and characterized regarding detailed structure. Microsomes were prepared from these cells and the activities of D-**glucuronyl** C5-**epimerase** and 4-O-sulfotransferase were determined Chondrocytes from nasal cartilage synthesized biglycan and decorin containing 10%, articular chondrocytes 20-30%, and fibroblast 80% of the uronosyl residues in the L-iduronyl configuration. All three tissues contained high amount of 4-O-sulfotransferase activity. The activity of D-**glucuronyl** C5- **epimerase** showed different relationships. Fibroblasts contained a high level of the epimerase activity, articular chondrocytes intermediary activity, and in nasal cartilage it was barely detectable. The data indicate that the activity of the D-**glucuronyl** C5- **epimerase** is the main factor for formation of dermatan sulfate in tissues. (c) 2001 Academic Press.

L11 ANSWER 10 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN
2000:108077 Document No. 132:262013 Biosynthesis of heparin/heparan sulfate: kinetic studies of the **glucuronyl** C5-**epimerase** with N-sulfated derivatives of the Escherichia coli K5 capsular polysaccharide as substrates. Hagner-McWhirter, Asa; Hannesson, Helgi H.; Campbell, Patrick; Westley, John; Roden, Lennart; Lindahl, Ulf; Li, Jin-Ping (Department of Medical Biochemistry and Microbiology, The Biomedical Center, Uppsala University, Uppsala, S-751 23, Swed.). Glycobiology, 10(2), 159-171 (English) 2000. CODEN: GLYCE3. ISSN: 0959-6658. Publisher: Oxford University Press.

AB The D-**glucuronyl** C5-**epimerase** involved in the biosynthesis of heparin and heparan sulfate was investigated with focus on its substrate specificity, its kinetic properties, and a comparison of epimerase prepns. from the Furth mastocytoma and bovine liver, which synthesize heparin and heparan sulfate, resp. New substrates for the epimerase were prepared from the capsular polysaccharide of Escherichia coli K5, which had been labeled at C5 of its D-glucuronic and N-acetyl-D-glucosamine moieties by growing the bacteria in the presence of D-[5-3H]glucose. Following complete or partial (.apprx.50%) N-deacetylation of the polysaccharide by hydrazinolysis, the free amino groups were sulfated by treatment with trimethylamine·SO3 complex, which yielded products that were recognized as substrates by the epimerase and released tritium from C5 of the D-glucuronyl residues upon incubation with the enzyme. Comparison of the kinetic properties of the two substrates showed that the fully N-sulfated derivative was the best substrate in terms of its Km value, which was significantly lower than that of its partially N-acetylated counterpart. The Vmax values for the E.coli polysaccharide derivs. were essentially the same but were both lower than that of the O-desulfated

[3H]heparin used in our previous studies. Surprisingly, the apparent Km values for all three substrates increased with increasing enzyme concentration The reason for this phenomenon is not entirely clear at present. Partially purified C5-epimerase prepns. from the Furth mastocytoma and bovine liver, resp., behaved similarly in terms of their reactivity towards the various substrates, but the variation in apparent Km values with enzyme concentration precluded a detailed comparison of their kinetic properties.

L11 ANSWER 11 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN

1998:709175 Document No. 129:327732 DNA sequence coding for a bovine **glucuronyl** C5-**epimerase** and a process for its production. Lindahl, Ulf; Li, Jin-Ping (Swed.). PCT Int. Appl. WO 9848006 A1 19981029, 26 pp. DESIGNATED STATES: W: AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ, DE, DE, DK, DK, EE, EE, ES, FI, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-SE703 19980417. PRIORITY: SE 1997-1454 19970418.

AB An isolated or recombinant DNA sequence coding for a mammalian, including human, **glucuronyl** C5-**epimerase** or a functional derivative thereof capable of converting D-glucuronic acid (GlcA) to L-iduronic acid (IdoA) is provided. The cDNA sequence isolated from bovine liver contains an open reading frame corresponding to 444 amino acid residues. Also provided are: a recombinant expression vector comprising such DNA sequence; a host cell transformed with such recombinant expression vector; a process for the manufacture of a **glucuronyl** C5-**epimerase** or functional derivative thereof capable of converting GlcA to IdoA, comprising cultivation of a cell-line transformed with such recombinant expression vector; and a **glucuronyl** C5-**epimerase** or functional derivative thereof prepared by such process.

L11 ANSWER 12 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN

1998:681181 Document No. 130:33808 Novel rkp gene clusters of Sinorhizobium meliloti involved in capsular polysaccharide production and invasion of the symbiotic nodule: the rkpK gene encodes a UDP-glucose dehydrogenase. Kereszt, Attila; Kiss, Erno; Reuhs, Bradley L.; Carlson, Russell W.; Kondorosi, Adam; Putnoky, Peter (Institute of Genetics, Biological Research Center, Hungarian Academy of Sciences, Szeged, H-6701, Hung.). Journal of Bacteriology, 180(20), 5426-5431 (English) 1998. CODEN: JOBAAY. ISSN: 0021-9193. Publisher: American Society for Microbiology.

AB The production of exopolysaccharide (EPS) was shown to be required for the infection process by rhizobia that induce the formation of indeterminate nodules on the roots of leguminous host plants. In Sinorhizobium meliloti (also known as Rhizobium meliloti) Rm41, a capsular polysaccharide (KPS) analogous to the group II K antigens of Escherichia coli can replace EPS during symbiotic nodule development and serve as an attachment site for the strain-specific bacteriophage ϕ16-3. The rkpA to -J genes in the chromosomal rkp-1 region code for proteins that are involved in the synthesis, modification, and transfer of an as-yet-unknown lipophilic mol. which might function as a specific lipid carrier during KPS biosynthesis. Here we report that with a phage ϕ16-3-resistant population obtained after random Tn5 mutagenesis, we have identified novel mutants impaired in KPS production by genetic complementation and biochem. studies. The mutations represent two novel loci, designated the rkp-2 and rkp-3 regions, which are required for the synthesis of rhizobial KPS. The rkp-2 region harbors two open reading frames (ORFs) organized in monocistronic transcription units. Although both genes

are required for normal lipopolysaccharide production, only the second one, designated rkpK, is involved in the synthesis of KPS. We have demonstrated that RkpK possesses UDP-glucose dehydrogenase activity, while the protein product of ORF1 might function as a UDP-**glucuronic acid epimerase**.

L11 ANSWER 13 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN

1998:74274 Document No. 128:240970 A major common trisulfated hexasaccharide core sequence, hexuronic acid(2-sulfate)-glucosamine(N-sulfate)-iduronic acid-N-acetylglucosamine-glucuronic acid-glucosamine(N-sulfate), isolated from the low sulfated irregular region of porcine intestinal heparin. Yamada, Shuhei; Yamane, Yukari; Tsuda, Hiromi; Yoshida, Keiichi; Sugahara, Kazuyuki (Department of Biochemistry, Kobe Pharmaceutical University, Kobe, 658, Japan). Journal of Biological Chemistry, 273(4), 1863-1871 (English) 1998. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB The major structure of the low sulfated irregular region of porcine intestinal heparin was investigated by characterizing the hexasaccharide fraction prepared by extensive digestion of the highly sulfated region with Flavobacterium heparinase and subsequent size fractionation by gel chromatog. Structures of a tetrasaccharide, a pentasaccharide, and eight hexasaccharide components in this fraction, which accounted for approx. 19% (weight/weight) of the starting heparin representing the major oligosaccharide fraction derived from the irregular region, were determined by chemical and enzymic analyses as well as 1H NMR spectroscopy. Five compds. including one penta- and four hexasaccharides had hitherto unreported structures. The structure of the pentasaccharide with a glucuronic acid at the reducing terminus was assumed to be derived from the reducing terminus of a heparin glycosaminoglycan chain and may represent the reducing terminus exposed by a tissue endo-β-glucuronidase involved in the intracellular post-synthetic fragmentation of macromol. heparin. Eight out of the 10 isolated oligosaccharides shared the trisaccharide sequence, -4IdceAα1-4Glc-NAcα1-4GlcAβ1-, and its reverse sequence, -4GlcAβ1-4GlcNAcα1-4IdceAα1-, was not found. The latter has not been reported to date for heparin/heparan sulfate, indicating the substrate specificity of the D-**glucuronyl** C-5 **epimerase**. Furthermore, seven hexasaccharides shared the common trisulfated hexasaccharide core sequence ΔHexA(2-sulfate)α1- 4GlcN(N-sulfate)α1-4IdceAα1-4Gl-cNAcα1-4GlcAβ1- 4GlcN(N-sulfate) which contained the above trisaccharide sequence (ΔHexA, IdceA, GlcN, and GlcA represent 4-deoxy-α-L-threo-hex- 4-enepyranosyluronic acid, L-iduronic acid, D-glucosamine, and D-glucuronic acid, resp.) and addnl. sulfate groups. The specificity of the heparinase used for preparation of the oligosaccharides indicates the occurrence of the common pentasulfated octasaccharide core sequence, -4GcN(N-sulfate)α1-4HexA(2-sulfate)1-4 GlcN(N-sulfate)α1- 4IdceAα1-4GlcNAcα1-4GlcAβ1-4 GlcN(N-sulfate)α1-4HexA(2-sulfate)1-, where the central hexasaccharide is flanked by GlcN(N-sulfate) and HexA(2-sulfate) on the nonreducing and reducing sides, resp. The revealed common sequence consisted a low sulfated trisaccharide representing the irregular region sandwiched by highly sulfated regions and should reflect the control mechanism of heparin biosynthesis.

L11 ANSWER 14 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN

1996:72306 Document No. 124:110772 Biosynthesis of dermatan sulfate. Defructosylated Escherichia coli K4 capsular polysaccharide as a substrate for the D-**glucuronyl** C-5 **epimerase**, and an indication of a two-base reaction mechanism. Hannesson, Helgi H.; Hagner-McWhirter, Asa; Tiedemann, Kerstin; Lindahl, Ulf; Malmstroem, Anders (Dep. Med. Physiological Chemistry, Univ. Uppsala, Uppsala, S-751 23, Swed.). Biochemical Journal, 313(2), 589-96 (English) 1996. CODEN: BIJOAK. ISSN:

0264-6021. Publisher: Portland Press.

AB The capsular polysaccharide from Escherichia coli K4 consists of a chondroitin {[GlcA(β1→3)GalNAc(β1→4)]n} backbone, to which β-fructofuranose units are linked to C-3 of D-glucuronic acid (GlcA) residues. Removal of the fructose units by mild acid hydrolysis provided a substrate for the GlcA C-5 epimerase, which is involved in the generation of L-iduronic acid (IdoA) units during dermatan sulfate biosynthesis. Incubation of this substrate with solubilized fibroblast microsomal enzyme in the presence of 3H2O resulted in the incorporation of tritium at C-5 of hexuronyl units. A Km of 67+10-6 M hexuronic acid (equivalent to disaccharide units) was determined, which is similar to that (80+10-6 M) obtained for dermatan (desulfated dermatan sulfate). Vmax. was about 4 times higher with dermatan than with the K4 substrate. A defructosylated K4 polysaccharide isolated after incubation of bacteria with D-[5-3H]glucose released 3H2O on reaction with the epimerase, and thus could be used to assay the enzyme. Incubation of a K4 substrate with solubilized microsomal epimerase for 6 h in the presence of 3H2O resulted in the formation of about 5% IdoA and approx. equal amts. of 3H in GlcA and IdoA. A corresponding incubation of dermatan yielded approx. 22% GlcA, which contained virtually all the 3H label. These results are tentatively explained in terms of a two-base reaction mechanism, involving a monoprotic L-ido-specific base and a polyprotic D-gluco-specific base. Most of the IdoA residues generated by the enzyme occurred singly, although some formation of two or three consecutive IdoA-containing disaccharide units was observed

L11 ANSWER 15 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN

1994:624763 Document No. 121:224763 Biosynthesis of heparin/heparan sulfate. Purification of the D-**glucuronyl** C-5 **epimerase** from bovine liver. Campbell, Patrick; Hannesoson, Helgi H.; Sandbaeck, Dagmar; Roden, Lennart; Lindahl, Ulf; Li, Jin-ping (Univ. Alabama, Birmingham, AL, 35294, USA). Journal of Biological Chemistry, 269(43), 26953-8 (English) 1994. CODEN: JBCHA3. ISSN: 0021-9258.

AB The D-**glucuronyl** C-5 **epimerase** involved in the biosynthesis of heparin/heparan sulfate was purified from the high speed supernatant fraction of a homogenate of bovine liver by chromatog. on immobilized O-desulfate heparin, red Sepharose, Ph Sepharose, and Con A-Sepharose. After close to 1 million-fold purification, 10-15% yield, the product gave a single band on SDS-PAGE with silver staining and had a mobility corresponding to an Mr of .apprx.52,000. Since the epimerase assay used in the course of purification was based on release of tritium, as [3H]H2O, from a [5-3H]uronyl-labeled substrate, it was important to establish that the purified enzyme did indeed catalyze the actual conversion of D-glucuronyl to L-iduronyl residues. Upon incubation of the purified enzyme with 3H-labeled heparosan N-sulfate, prepared by metabolic labeling (with D-[1-3H]glucose) of a capsular polysaccharide from Escherichia coli K5 and subsequent chemical partial N-deacetylation and N-sulfation, approx. 30% of the D-glucuronyl residues located between two N-sulfated glucosamine units were converted to L-iduronyl units.

L11 ANSWER 16 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN

1982:541943 Document No. 97:141943 Histochemical studies on the enzymes of Hymenolepis diminuta (Rud., 1819) (Cestoda). XIV. Uridinediphosphoglucose (UDPG) dehydrogenase, UDPG epimerase and UDP-**glucuronate epimerase**. Moczon, Tadeusz (Inst. Parasitol., Pol. Acad. Sci., Warsaw, 00-973, Pol.). Acta Parasitologica Polonica, 28(12-24), 187-96, 3 plates (English) 1981. CODEN: APRPAX. ISSN: 0065-1478.

AB By histochem. methods, the 3 title enzymes were detected in tissues of mature H. diminuta. Their activity was highest in the subtegumental parenchyma, indicating that this is where precursors of mucopolysaccharides are

synthesized which enter into mucosubstances coating the cestode body. During embryogenesis of H. diminuta, all 3 enzymes were found mainly in macromeres, suggesting that these cells synthesize mucoproteins of the inner envelope of the oncosphere. The cestode is able to synthesize UDP-glucuronate, which is required for biosynthesis of glucuronides. None of the enzymes was found in cells in invasive oncospheres and cysticercoids.

L11 ANSWER 17 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN

1977:103846 Document No. 86:103846 Studies on cartilage formation. XX. Histochemical investigation of some enzymes of glycogen metabolism in regenerative articular surfaces. Hadhazy, C.; Glant, T.; Mandi, B.; Harmati, S.; Bordan, L.; Balogh, K. (Inst. Anat., Histol. Embryol., Univ. Med. Sch., Debrecen, Hung.). Acta Morphologica Academiae Scientiarum Hungaricae, 23(3), 183-93 (English) 1976. CODEN: AMSHAR. ISSN: 0001-6217.

AB The regenerating articular surface of the canine femur distal articular cartilage consisted of fibrous tissue and cartilage islets; the latter contained cells differentiating into cartilage and young chondrocytes. Glycolytic enzyme activity was observed in the cells of the regenerating articular surface. The activity of hexokinase and aldolase was substantially greater, and that of phosphohexose isomerase was slightly greater in the connective tissue than in the cartilage islets. Glyceraldehyde 3-phosphate dehydrogenase and lactate dehydrogenase (LDH) showed maximum activity in all cell types. The activity of LDH M-isoenzymes (LDH5 and LDH4) was predominate on the entire articular surface, and the activity of H-isoenzymes (LDH1 and LDH2) was low in the connective tissue regions. Glucose 6-phosphatase and fructose 1,6-diphosphatase activities were limited to the cartilage islets; the latter enzyme was detected only in the chondroblasts and the young chondrocytes. Phosphoglucomutase and UDP-glucose dehydrogenase activities were similar between the connective tissue and the cartilage islets, whereas UDP-**glucuronate epimerase** was restricted to the chondroblasts and the young chondrocytes. No phosphorylase activity was found, whereas glycogen synthetase activity was present in the cartilage islets.

L11 ANSWER 18 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN

1973:68596 Document No. 78:68596 Purification and properties of udp-glucuronic acid 4-epimerase of Anabaena flosaquae. Gaunt, Mary Alice (Marquette Univ., Milwaukee, WI, USA). 112 pp. Avail. Univ. Microfilms, Ann Arbor, Mich., Order No. 72-20,388 From: Diss. Abstr. Int. B 1972, 33(1), 42 (English) 1971.

AB Unavailable

L11 ANSWER 19 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN

1971:548198 Document No. 75:148198 Effect of 17β estradiol on the metabolism of chondroitin sulfate B. V. Effect of 17β estradiol on the protein content and composition of the uridine diphosphate-D-glucuronic acid-5-epimerase-containing protein fraction of rat skin. Nordbo, H. (Dent. Fac., Univ. Oslo, Oslo, Norway). Steroidologia, 1(6), 362-6 (English) 1970. CODEN: STDLB8. ISSN: 0049-2221.

GI For diagram(s), see printed CA Issue.

AB 17β-Estradiol (I) (4 mg/day for 6 and 12 days) decreased the skin protein content in male rats by 1.3 and 7%, resp.; 40 μg doses caused decreases of 0.06 and 1.5%, resp., and 4 μg doses had no effect. Caloric undernourishment had no essential influence on the skin protein content. Starch gel electrophoresis revealed significant changes in the composition of skin proteins after I treatment; 1 or 2 zones with weak UDP-D-glucuronic acid 5-

epimerase activity, seen with normal skin, were not seen after massive I doses.

L11 ANSWER 20 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN

1971:537228 Document No. 75:137228 Effect of 17β-estradiol on the metabolism of chondroitin sulfate B. IV. Effect of 17β-estradiol on the activity of uridine diphosphate-D-glucuronic acid-5-epimerase in rat skin. Nordbo, H. (Dent. Fac., Univ. Oslo, Oslo, Norway). Steroidologia, 1(6), 356-61 (English) 1970. CODEN: STDLB8. ISSN: 0049-2221.

GI For diagram(s), see printed CA Issue.

AB 17β-Estradiol (I) (4 mg/day for 6 and 12 days) decreased the activity of UDP-D-glucuronic acid 5-epimerase in the skin of male rats by 37-38 and 54-55%, resp.; 40 μg doses caused decreases of 17-18 and 26-27%, resp., and 4 μg doses had no effect. I had no direct effect on the enzyme activity in vitro.

L11 ANSWER 21 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN

1969:409792 Document No. 71:9792 UDP-D-glucuronate 4-epimerase in blue-green algae. Ankel, Helmut; Tischer, Robert G. (Marquette Sch. of Med., Milwaukee, WI, USA). Biochimica et Biophysica Acta, 178(2), 415-19 (English) 1969. CODEN: BBACAQ. ISSN: 0006-3002.

AB The existence of uridine 5'-(α-D-glucopyranosyluronic acid pyrophosphate) (I) 4-epimerase was demonstrated in Anabaena flos-aquae, a member of the Nostoc family. Cultures were grown on modified Knop medium. I epimerase activity was precipitated from crude exts. of the algae with (NH4)2SO4, between 1.7 and 3.3M. The precipitated protein was dissolved in buffer and dialyzed against buffer. Incubation of 14C-labeled I with the enzyme preparation followed by paper electrophoresis yielded a band which was characterized as uridine 5'-(α-D-galactopyranosyluronic acid pyrophosphate) (II). The epimerization of II was shown by incubating radioactive II with Anabaena enzyme, and identifying the products electrophoretically. The enzyme catalyzed the epimerization of I to the same extent in the absence and presence of 2mM NAD, and was not inhibited by 2mM NADH.

L11 ANSWER 22 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN

1969:112934 Document No. 70:112934 Two particular histoenzymological aspects at the Purkinje cell level in the cerebellum. Onicescu, Doina; Cuida, Ioana (Fac. Med., Bucharest, Rom.). Journal of Neurochemistry, 16(3), 467 (English) 1969. CODEN: JONRA9. ISSN: 0022-3042.

AB The reaction for UDP glucuronate 4-epimerase (EC 5.1.3.6) (Diculescu, 1965) was strongly pos. in the perikaryon of the Purkinje cells of the cerebellar cortex (species not specified). The reaction was neg. in the dendritic and axonal processes and in all the other structures of the cerebellar cortex. The presence of epimerase activity in the neurons indicate the metabolism of nucleotide-bound hexoses at the Purkinje cell level and shows that the required enzyme is present for production of galacturonic and (or) iduronic acid, which is necessary for the synthesis of mucopolysaccharide in the nerve cell. An intense reaction was found for Mg2+-activated ATPase (EC 3.6.1.3) in the perikaryon of the Purkinje cells. An intense pos. ATPase reaction appeared in numerous nerve fibers, especially in those localized in the mol. layer surrounding the body of the Purkinje cells.

L11 ANSWER 23 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN

1968:501 Document No. 68:501 UDP-D-Glucuronic acid 5-epimerase frrom rabbit skin. Davidson, Eugene Abraham (Duke Univ. Med. Center, Durham, NC, USA). Methods in Enzymology, 8, 281-4 (English) 1966. CODEN: MENZAU. ISSN:

0076-6879.

AB UDP-D-glucuronic acid 5-epimerase was partially purified from rabbit skin and was shown to require DPN. The enzyme was inhibited by DPNH, and TPN could not be substituted for DPN.

L11 ANSWER 24 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN

1967:63142 Document No. 66:63142 Histochemistry of epimerases. I. Uridine diphosphate **glucuronic acid epimerases.** Diculescu, Ilie; Onicescu, Doina (Inst. Med. Pharm., Bucharest, Rom.). Acta Histochemica, 25(5-8), 242-50 (English) 1966. CODEN: AHISA9. ISSN: 0065-1281.

AB Connective tissue cells and basement membranes from laboratory animals reduced Nitro Blue Tetrazolium in the presence of uridine diphosphoglucuronic acid (UDPGA) (at pH 7.1-7.2). The histochem. reaction occurred in the presence of NAD and low MgCl2 and EDTA concns. p-Chloromercuribenzoate inhibited the reaction, while cysteine prevented inhibition. The histochem. localization of UDPGA epimerases differs from that of uridine diphosphoglucose dehydrogenase, diaphorases, beta-glucuronidases, and alpha-galactosidases.

L11 ANSWER 25 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN

1966:85908 Document No. 64:85908 Original Reference No. 64:16204h,16205a-b Histochemical demonstration of uridine diphosphate-D-**glucuronic acid epimerases.** Diculescu, Ilie; Onicescu, Doina (Fac. Med., Bucharest, Rom.). Histochemie, 5(5), 450-2 (French) 1965. CODEN: HICHAU. ISSN: 0018-2222.

AB Using UDP-GA (UDP-glucuronic acid) as substrate, histochem. tests were made on UDP-GA-4-epimerase and UDP-GA-5-epimerase. Fresh-frozen tissue sections from various sources were buffered to pH 7.1-7.2 and incubated at 37° for 30-45 min. In all tissues the most pos. enzymic reactions were found in the conjunctive cells. Moderately intensive reactions were observed in the hepatocytes, in smooth and striated muscle fibers, and in cardiac muscle. Kidney glomerules were always neg., and the tubules showed varying intensities. The Kupffer's cells of the hepatocytes reacted very clearly, p-Chloromercuri-benzoic acid (PCMB) at 0.02μM concentration completely inhibited the reaction. Cysteine at 0.4μM concentration prevented the inhibiting. action of PCMB only in basal membranes and conjunctive cells, but was without effect in liver cells, muscle fibers, and nephrocytes. Conjunctive cells and basal membrane contain an enzyme specific for UDP-GA epimerases. This histochem. demonstration supports the hypothesis that an electron transfer occurs during epimerization.

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=> S EPIMERASE
          2188 EPIMERASE
           260 EPIMERASES
L12        2229 EPIMERASE
                (EPIMERASE OR EPIMERASES)

=> S N TERMIN?
       2684375 N
        487346 TERMIN?
L13      95910 N TERMIN?
                (N(W)TERMIN?)

=>
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=> S L12 AND L13
L14          73 L12 AND L13

=> S L12(6A)L13
L15          12 L12(6A)L13

=> D 1-12 TI
=> D L15 2 CBIB ABS

L15  ANSWER 2 OF 12  CAPLUS  COPYRIGHT 2004 ACS on STN
2002:449840   Document No. 137:29828  Cloning and characterization of mouse
      glucuronyl C5-epimerase, and use of N-terminal
      fragments of the epimerase in fusion protein constructs.
      Jalkanen, Markku; El Darwish, Kamel; Lindahl, Ulf; Li, Jin-Ping (Biotie
      Therapies Corp., Finland).  PCT Int. Appl. WO 2002046379 A2 20020613, 58
      pp.  DESIGNATED STATES: W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR,
      BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB,
      GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK,
      LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL,
      PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN,
      YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ,
      CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC,
      ML, MR, NE, NL, PT, SE, SN, TD, TG, TR.  (English).  CODEN: PIXXD2.
      APPLICATION: WO 2001-FI1068 20011207.  PRIORITY: US 2000-PV304180
      20001208.
AB    The invention is directed to a novel purified mouse glucuronyl C5-epimerase,
      fragments thereof, nucleic acids encoding the same and the recombinant
      production thereof.  A mouse liver gene encoding glucuronyl C5-epimerase was
      cloned.  The cDNA sequence and the encoded amino acid sequence of the mouse
      liver C5-epimerase are provided.  The amino acid sequence of the C5-epimerase
      was found to be 618 amino acids long, with a mol. weight of 71.18 kDa.  The
      invention is also directed to fragments of such epimerase, especially N-
      terminal fragments that are useful in fusion protein constructs to enhance the
      activity of recombinantly-produced heterologous epimerase enzymes.


=> E JALKANEN M/AU
=> S E3-E11
            12 "JALKANEN M"/AU
             1 "JALKANEN M M"/AU
             1 "JALKANEN M T"/AU
             1 "JALKANEN MAKKU"/AU
             2 "JALKANEN MARITA"/AU
            93 "JALKANEN MARKKU"/AU
             1 "JALKANEN MARKKU T"/AU
             2 "JALKANEN MARKKU TAPANI"/AU
             1 "JALKANEN MARKKUS"/AU
L16        114 ("JALKANEN M"/AU OR "JALKANEN M M"/AU OR "JALKANEN M T"/AU OR
               "JALKANEN MAKKU"/AU OR "JALKANEN MARITA"/AU OR "JALKANEN MARKKU"
               /AU OR "JALKANEN MARKKU T"/AU OR "JALKANEN MARKKU TAPANI"/AU OR
               "JALKANEN MARKKUS"/AU)

=> E DARWISH K/AU
=> S E3,E7
             2 "DARWISH K"/AU
             5 "DARWISH KAMEL"/AU
L17          7 ("DARWISH K"/AU OR "DARWISH KAMEL"/AU)
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=> E LINDAHL U/AU
=> S E3-E5
             19 "LINDAHL U"/AU
            232 "LINDAHL ULF"/AU
              1 "LINDAHL ULF PER FREDRIK"/AU
L18         252 ("LINDAHL U"/AU OR "LINDAHL ULF"/AU OR "LINDAHL ULF PER FREDRIK"
                /AU)

=> E LI J/AU
=> S E3,E27-E30
           1592 "LI J"/AU
             77 "LI J P"/AU
              2 "LI J P L"/AU
              1 "LI J PENGAMAN"/AU
              3 "LI J PENGMAN"/AU
L19        1675 ("LI J"/AU OR "LI J P"/AU OR "LI J P L"/AU OR "LI J PENGAMAN"/AU
                OR "LI J PENGMAN"/AU)

=> E LI JIN/AU
=> S E3,E61,E62
            485 "LI JIN"/AU
              2 "LI JIN PENG"/AU
             26 "LI JIN PING"/AU
L20         513 ("LI JIN"/AU OR "LI JIN PENG"/AU OR "LI JIN PING"/AU)

=> S L16,L17,L18,L19,L20
L21        2542 (L16 OR L17 OR L18 OR L19 OR L20)

=> S L21 AND L12
L22          24 L21 AND L12

=> S L22 NOT L7
L23          13 L22 NOT L7

=> D 1-13 CBIB ABS

L23  ANSWER 1 OF 13  CAPLUS  COPYRIGHT 2004 ACS on STN
2001:526630  Document No. 135:329725  Molecular diversity of heparan sulfate.
     Esko, Jeffrey D.; Lindahl, Ulf (Department of Cellular and
     Molecular Medicine, Glycobiology Research and Training Center, University
     of California, San Diego, La Jolla, CA, 92093-0687, USA). Journal of
     Clinical Investigation, 108(2), 169-173 (English) 2001. CODEN: JCINAO.
     ISSN: 0021-9738. Publisher: American Society for Clinical Investigation.
AB   A review, with refs., on the structure, function, and biosynthesis of heparan
     sulfate (HS). The relationship between structure and function of the HS mols.
     and how altered HS biosynthesis and catabolism can lead to human disorders are
     also discussed.


L23  ANSWER 2 OF 13  CAPLUS  COPYRIGHT 2004 ACS on STN
2000:270488  Document No. 133:27957  Biosynthesis of heparin/heparan sulphate:
     mechanism of epimerization of glucuronyl C-5. Hagner-McWhirter, Asa;
     Lindahl, Ulf; Li, Jin-Ping (Department of Medical
     Biochemistry and Microbiology, Section for Medical Biochemistry,
     Biomedical Center, University of Uppsala, Uppsala, SE-751 23, Swed.).
     Biochemical Journal, 347(1), 69-75 (English) 2000. CODEN: BIJOAK. ISSN:
     0264-6021. Publisher: Portland Press Ltd..
AB   In the biosynthesis of heparin and heparan sulfate, D-glucuronic acid residues
     are converted into L-iduronic acid (IdoA) units by C-5 epimerization, at the
     polymer level. The reaction catalyzed by the epimerase occurs by reversible
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abstraction and readdn. of a proton at C-5 of target hexuronic acid residues, through a carbanion intermediate, with or without an inversion of configuration at C-5. Incubation of chemical N-sulfated capsular polysaccharide from Escherichia coli K5 ([4GlcAβ1-4GlcNSO3α1-]n), or of O-desulfated heparin (predominantly [4IdoAα1-4GlcNSO3α1-]n) with purified C-5 **epimerase** from bovine liver, resulted in the interconversion of glucuronic acid and IdoA residues, which reached equilibrium (30-40% IdoA/total hexuronic acid) after approx. 1 h of incubation. Similar incubations performed in the presence of 3H2O resulted in progressive labeling at C-5 of the target hexuronic acid units of either substrate polysaccharide. Contrary to chemical D-gluco/L-ido equilibrium, established within 1 h of incubation, the accumulation of 3H label continued for at least 6 h. This isotope effect suggests that the second stage of the reaction, i.e. the re-addition of a proton to the carbanion intermediate, is the rate-limiting step of the overall process. Anal. of the 5-3H-labeled polysaccharide products showed that the 3H was approx. equally distributed between glucuronic acid and IdoA units, irresp. of incubation time (from 15 min to 72 h) and of the relative proportions of the two epimers in the substrate. This finding points to a catalytic mechanism in which the abstraction and re-addition of C-5 protons are effected by two polyprotic bases, presumably lysine residues. Previous expts. relating to the biosynthesis of dermatan sulfate were similarly interpreted in terms of a two-base epimerization mechanism but differed from the present findings by implicating one monoprotic and one polyprotic base function.

L23 ANSWER 3 OF 13 CAPLUS COPYRIGHT 2004 ACS on STN

1997:673314 Document No. 127:325826 Heparan sulfate - a polyanion with multiple messages. **Lindahl, Ulf** (Dept. of Medical and Physiological Chemistry, University of Uppsala, Uppsala, S-751 23, Swed.). Pure and Applied Chemistry, 69(9), 1897-1902 (English) 1997. CODEN: PACHAS. ISSN: 0033-4545. Publisher: Blackwell.

AB A review with 29 refs. Proteoglycans are composed of sulfate-substituted, neg. charged glycosaminoglycan chains that are covalently linked to proteins. Studies on proteoglycan biosynthesis have been focused on the isolation and mol. cloning of the various enzymes that catalyze this process. Enzymes involved in the biosynthesis of heparin and heparan sulfate include the glycosyltransferases responsible for generating the initial (GlcA-GlcNAc)n chains, the GlcNAc N-deacetylase/N-sulfotransferase that introduces N-sulfate groups, the D-GlcA C5-**epimerase** that generates L-IdoA units, and O-sulfotransferases that sulfate hydroxyl groups in various positions. Restricted polymer modification will lead to the generation of complex saccharide sequences of varied structure. Attempts have been made to define the minimal saccharide sequences required for binding of various proteins of biol. interest, including growth factors of the fibroblast growth factor family. It is proposed that many "heparin-binding proteins", with affinity for the predominant structure in the highly sulfated heparin mol., may bind to distinct, less modified, regions of heparan sulfate chains. These studies are expected to promote our understanding of the regulatory mechanisms behind polysaccharide biosynthesis, and of the physiol. roles of proteoglycans. Further, they may provide the basis for the generation of novel drugs.

L23 ANSWER 4 OF 13 CAPLUS COPYRIGHT 2004 ACS on STN

1996:334946 Document No. 125:87062 Biologically active, heparan sulfate-like species by combined chemical and enzymic modification of the Escherichia coli polysaccharide K5. Casu, Benito; Grazioli, Giordana; Hannesson, Helgi H.; Jann, Barbara; Jann, Klaus; **Lindahl, Ulf**; Naggi, Annamaria; Oreste, Pasqua; Razi, Nahid; et al. (Ist. Chim. Biochim. G. Ronzoni, Milan, Italy). Carbohydrate Letters, 1(2), 107-114 (English)

1994. CODEN: CLETEC. ISSN: 1073-5070. Publisher: Harwood.

AB Semi-synthetic heparan sulfate-like glycosaminoglycans have been prepared from the E. coli K5 polysaccharide, by controlled N-deacetylation (with hydrazine), followed by N-sulfation (with trimethylamine.SO3), partial C-5-epimeriazation (with a purified C-5 **epimerase**), and O-sulfation (with pyridine.SO3, and with a crude 3-O-sulfotransferase). The in vitro inhibition of activated Factor X by antithrombin of the end-products is similar to that of beef mucosal heparan sulfate.

L23 ANSWER 5 OF 13 CAPLUS COPYRIGHT 2004 ACS on STN

1991:490186 Document No. 115:90186 Decreased activity of the heparan sulfate-modifying enzyme glucosaminyl N-deacetylase in hepatocytes from streptozotocin-diabetic rats. Unger, Erik; Pettersson, Inger; Eriksson, Ulf J.; **Lindahl, Ulf**; Kjellen, Lena (Dep. Vet. Med. Chem., Swed. Univ. Agric. Sci., Uppsala, S-751 23, Swed.). Journal of Biological Chemistry, 266(14), 8671-4 (English) 1991. CODEN: JBCHA3. ISSN: 0021-9258.

AB N-Deacetylation is the initial polymer modification step in heparan sulfate biosynthesis and a prerequisite to subsequent N- and O-sulfation. It has previously been shown that the sulfation of liver heparan sulfate is lowered in diabetes (Kjellen, L., et al., 1983). To investigate whether the reduced sulfation is the result of a lowered N-deacetylase activity, this enzyme was assayed in hepatocytes from streptozotocin- diabetic rats. In addition, the activity of the glucuronosyl C5- **epimerase**, which catalyzes a modification reaction subsequent to N-sulfation, was measured. The deacetylase activity, expressed per μg of cell protein, was about 40% lower in diabetic hepatocytes as compared with control cells, whereas the **epimerase** activity was unaffected. Recently, a .apprx.110-kDa glycoprotein that carries N-sulfotransferase activity was identified as one of at least two protein components required for N-deacetylation in mouse mastocytoma tissue (Pettersson, I., et al, 1991). The authors, therefore investigated if the lowered N-deacetylase activity in diabetes could be ascribed to a deficiency in either one of the corresponding rat components. The results indicated that (i) the glycoprotein component is present in limiting amts. in both control and diabetic cells, (ii) diabetes results in a lowered activity of this component, and (iii) excess amts. of the addnl. protein(s) needed for N-deacetylase activity are presented in both control and diabetic cells.

L23 ANSWER 6 OF 13 CAPLUS COPYRIGHT 2004 ACS on STN

1991:201865 Document No. 114:201865 Biosynthesis of heparin. Use of Escherichia coli K5 capsular polysaccharide as a model substrate in enzymic polymer-modification reactions. Kusche, Marion; Hannesson, Helgi H.; **Lindahl, Ulf** (Biomed. Cent., Swed. Univ. Agric. Sci., Uppsala, S-751 23, Swed.). Biochemical Journal, 275(1), 151-8 (English) 1991. CODEN: BIJOAK. ISSN: 0306-3275.

AB A capsular polysaccharide from E. coli K5 has the same structure [-(4)βGlcA(1)→(4)αGlcNAc(1)-]n, as that of the nonsulfated precursor polysaccharide in heparin biosynthesis. The K5 polysaccharide was N-deacetylated (by hydrazinolysis) and N-sulfated, and was then incubated with detergent-solubilized enzymes from a heparin-producing mouse mastocytoma, in the presence of adenosine 3'-phosphate 5'-phospho[35S]sulfate ([35S]PAPS). Structural anal. of the resulting 35S-labeled polysaccharide revealed the formation of all the major disaccharide units found in heparin. The identification of 2-O-[35S]sulfated IdoA (L-iduronic acid) as well as 6-O-[35S]sulfated GlcNSO3 units demonstrated that the modified K5 polysaccharide served as a substrate in the hexuronosyl C-5-**epimerase** and the major O-sulfotransferase reactions involved in the biosynthesis of heparin. The GlcA units of the native (N-acetylated) E. coli polysaccharide were attacked by the

epimerase only when PAPS was present in the incubations, whereas those of the chemical N-sulfated polysaccharide were epimerized also in the absence of PAPS, in accord with the notion that N-sulfate groups are required for epimerization. With increasing concns. of PAPS, the mono-O-sulfated disaccharide unit -IdoA(2-OSO3)-GlcNSO3- was progressively converted into the di-O-sulfated species -IdoA(2-OSO3)-GlcNSO3(6-OSO3)-. A small proportion of the 35S-labeled polysaccharide was found to bind with high affinity to the proteinase inhibitor antithrombin. This proportion increased with increasing concentration of PAPS up to a level corresponding to .apprx.1-2% of the total incorporated 35S. The solubilized enzymes thus catalyzed all the reactions required for the generation of functional antithrombin-binding sites.

L23 ANSWER 7 OF 13 CAPLUS COPYRIGHT 2004 ACS on STN

1984:63972 Document No. 100:63972 Biosynthesis of heparin. Substrate specificity of heparosan N-sulfate D-glucuronosyl 5-**epimerase**. Jacobsson, Ingvar; **Lindahl**, **Ulf**; Jensen, John W.; Roden, Lennart; Prihar, Harry; Feingold, David S. (Biomed. Cent., Swed. Univ. Agric. Sci., Uppsala, S-751 23, Swed.). Journal of Biological Chemistry, 259(1), 1056-63 (English) 1984. CODEN: JBCHA3. ISSN: 0021-9258.

AB The substrate specificity of heparosan N-sulfate D-glucuronosyl 5- **epimerase** (I) from a mouse mastocytoma was examined to determine the effects of N-Ac and O-sulfate groups on substrate recognition by I. [5-3H]glucuronosyl-labeled heparosan N-sulfate was prepared enzymically and was modified chemical by partial N-desulfation and N-acetylation. After enzymic release of 3H, the location of remaining label was determined by deaminative cleavage and anal. of resulting di-, tetra-, and higher oligosaccharides. This anal. indicated that a D-glucuronosyl residue is recognized as a substrate if it is linked at C-1 to an N-acetylated glucosamine residue and at C-4 to an N-sulfated unit. However, the reverse structure, in which the D-glucuronosyl moiety is bound at C-1 to an N-sulfated residue and at C-4 to N-acetylated glycosamine, is not a substrate. Similar studies with O-sulfated heparin intermediates showed that O-sulfate groups either at C-2 of the L-iduronosyl moieties or at C-6 of vicinal D-glucosaminyl moieties prevent 5-epimerization. These findings were confirmed by studies of the reverse reaction, in which 3H was incorporated from 3H2O into partially O-desulfated heparin and the location of incorporated radioactivity was determined These and more direct expts. corroborated the previous conclusion that the L-iduronosyl moieties are formed after N-sulfation, but before O-sulfation. Assessment of the influence of substrate size on the reaction further showed that a large substrate is preferred; an octasaccharide released 3H at a rate .apprx.10% of that observed for the parent polysaccharide, and some release occurred also with smaller oligosaccharides.

L23 ANSWER 8 OF 13 CAPLUS COPYRIGHT 2004 ACS on STN

1983:434866 Document No. 99:34866 Biosynthesis of heparin. A new substrate for heparosan-N-sulfate-D-glucopyranosyluronate 5-**epimerase**. Jensen, John W.; Roden, Lennart; Jacobsson, Ingvar; **Lindahl**, **Ulf** ; Prihar, Harry; Feingold, David S. (Sch. Med. Dent., Univ. Alabama, Birmingham, AL, 35294, USA). Carbohydrate Research, 117, 241-53 (English) 1983. CODEN: CRBRAT. ISSN: 0008-6215.

AB New substrates were prepared for the determination of heparosan-N-sulfate-D-glucopyranosyluronate 5-**epimerase**, which catalyzes formation of L-iduronic acid residues in the course of heparin biosynthesis. Heparin and heparan sulfate were chemical modified by desulfation in aqueous Me2SO, deacetylation by hydrazinolysis, and N-sulfation with SO3-Me3N complex. The modified polysaccharides were incubated with partially purified **epimerase** from bovine liver in the presence of 3H2O to incorporate 3H into both D-gluco- and L-idopyranosyluronate residues. Incubation of the labeled polysaccharides with

liver **epimerase** released 3H. The complete release of radioactivity after exhaustive incubation indicated that the 3H atom was located at C-5 of the uronate residues. Under appropriate conditions, the release was linear with time and enzyme concentration; Km values of .apprx.20 mM (expressed as uronic acid concentration) were determined for both the heparin- and the heparan sulfate-derived substrates. In contrast to the modified polysaccharides, unmodified heparin did not incorporate significant amts. of radioactivity when exposed to 3H2O in the presence of **epimerase**.

L23 ANSWER 9 OF 13 CAPLUS COPYRIGHT 2004 ACS on STN

1980:490918 Document No. 93:90918 Biosynthesis of heparin. Concerted action of late polymer-modification reactions. Jacobsson, Ingvar; **Lindahl, Ulf** (Fac. Vet. Med., Swed. Univ. Agric. Sci., Uppsala, S-751 23, Swed.). Journal of Biological Chemistry, 255(11), 5094-100 (English) 1980. CODEN: JBCHA3. ISSN: 0021-9258.

AB The substrate specificity of O-sulfotransferases involved in the biosynthesis of heparin was studied by incubating exogenous polysaccharide acceptors with mouse mastocytoma microsomal fraction in the presence of phosphoadenylyl sulfate-35S. Characterization of the labeled products showed that O-sulfation occurs preferentially in the vicinity of N-sulfate groups; that 2-O-sulfation of L-iduronic acid residues occurs preferentially or exclusively in the absence of a 6-O-sulfate group on adjacent D-glucosamine units; and that 6-O-sulfation of D-glucosamine residues occurs readily in the presence of 2-O-sulfate groups on adjacent L-iduronic acid units. Furthermore, structural anal. of microsomal heparin-precursor polysaccharides showed a distinct intermediate species that contained 2-O-sulfated L-iduronic acid units but essentially no 6-O-sulfate groups on the (N-sulfated) D-glucosamine residues. The results suggest that 2-O-sulfation of L-iduronic acid units is tightly coupled to the formation of these units (by 5-epimerization of D-glucuronic acid residues) and, furthermore, that both processes are completed before 6-O-sulfation of the polysaccharide mol. is initiated. D-Glucuronosyl 5-epimerization not accompanied by 2-O-sulfation occurs at a still earlier stage of polymer modification; the resulting L-iduronic acid units appear to remain nonsulfated throughout the subsequent modification reactions.

L23 ANSWER 10 OF 13 CAPLUS COPYRIGHT 2004 ACS on STN

1980:402857 Document No. 93:2857 Biosynthesis of heparin. VIII. Partial purification of the uronosyl C-5 **epimerase**. Malmstroem, Anders; Roden, Lennart; Feingold, David S.; Jacobsson, Ingvar; Baeckstroem, Gudrun; **Lindahl, Ulf** (Inst. Dent. Res., Univ. Alabama, Birmingham, AL, 35294, USA). Journal of Biological Chemistry, 255(9), 3878-83 (English) 1980. CODEN: JBCHA3. ISSN: 0021-9258.

AB Heparosan N-sulfate D-glucuronosyl 5-**epimerase**, which catalyzes the conversion of β-D-glucuronosyl to α-L-iduronosyl residues in the course of heparin biosynthesis, was purified .apprx.9000-fold from the high-speed supernatant fraction of a homogenate of a mouse mastocytoma. Following (NH4)2SO4 fractionation, the material precipitating between 35 and 60% saturation was subjected to a series of affinity chromatog. steps on matrixes containing immobilized concanavalin A, heparan sulfate, O-desulfated heparin, and Cibacron blue, resp. **Epimerase** purified by this procedure yielded 2 major components on Na dodecyl sulfate-polyacrylamide gel electrophoresis. The purified enzyme had approx. the same Kav (partition coefficient between liquid and gel phases) as bovine serum albumin when chromatographed on Sepharose 6B. The activity of the purified enzyme was increased 50-fold by addition of the fraction which was not adsorbed to concanavalin A-Sepharose. The stimulating factor is probably a protein, since it was nondialyzable and heat labile, and lost activity on digestion with trypsin.

L23 ANSWER 11 OF 13 CAPLUS COPYRIGHT 2004 ACS on STN
1980:89853 Document No. 92:89853 Biosynthesis of heparin. Hydrogen exchange at carbon 5 of the glycuronosyl residues. Prihar, Harry S.; Campbell, Patrick; Feingold, David Sidney; Jacobsson, Ingvar; Jensen, John W.; **Lindahl, Ulf**; Roden, Lennart (Sch. Med., Univ. Pittsburgh, Pittsburgh, PA, 15261, USA). Biochemistry, 19(3), 495-500 (English) 1980. CODEN: BICHAW. ISSN: 0006-2960.

AB O-Desulfated heparin is known to incorporate 3H into its glycosyluronic acid moieties when incubated in 3H2O with bovine liver heparosan-N-sulfate D-glucuronosyl 5-**epimerase** (I). The location of the incorporated 3H was established as follows: L-idosan-3H and a mixture of D-glucose-3H and L-idose-3H were isolated from enzymically tritiated O-desulfated heparin by esterification of the carboxyl groups with 1-ethyl-3-[3-(dimethylamino)propyl]carbodimide, reduction with NaBH4, acid hydrolysis, and deaminative cleavage. When the mixture of the 2 hexoses was converted to 3H-labeled Me glycosides and subsequently degraded by successive periodate oxidation, hypobromite oxidation, and acid hydrolysis, the only 3H-labeled fragment was glyceric acid. The same result was obtained upon degradation of L-idosan-3H, indicating that in both the D-glucuronic acid and L-iduronic acid residues, 3H was located at C-5. In another approach, D-glucuronic acid-3H was isolated from the 3H-labeled polysaccharide and was converted to Me (Me D-glucopyranosid)uronate. On reduction with NaBH4 in anhydrous MeOH, this compound lost its radioactivity and yielded unlabeled Me α-D-glucopyranoside. This finding indicates that the label was located at C-5, since it was previously shown that the H atom in this position is completely exchanged under the reaction conditions used and that H atoms in other positions are not affected. Thus, incubation of O-desulfated heparin in 3H2O with I introduces 3H at C-5 of the glycuronosyl moieties of the substrate and no exchange of H atoms at C-2, C-3, or C-4 with protons of the medium or with the C-5 H atom occurs during the reaction.

L23 ANSWER 12 OF 13 CAPLUS COPYRIGHT 2004 ACS on STN
1979:606117 Document No. 91:206117 Biosynthesis of heparin: tritium incorporation into chemically modified heparin catalyzed by C-5-uronosylepimerase. Jensen, John; Campbell, Patrick; Roden, Lennart; Jacobsson, Ingvar; Baeckstroem, Gudrun; **Lindahl, Ulf** (USA). Glycoconjugate Res., Proc. Int. Symp., 4th, Meeting Date 1977, Volume 2, 713-17. Editor(s): Gregory, John D.; Jeanloz, Roger W. Academic: New York, N. Y. (English) 1979. CODEN: 41RSAU.

AB Partially purified uronosylepimerase from bovine liver catalyzed the incorporation of 3H from 3H2O into chemical modified heparin. Similar results were obtained with modified heparan sulfate from hog mucosa and on incubation of the microsomal fraction of the Furth mastocytoma with modified heparan sulfate from aorta. The treated compds. were used as substrates for the C-5-uronosylepimerase involved in heparin biosynthesis using a tritium release assay.

L23 ANSWER 13 OF 13 CAPLUS COPYRIGHT 2004 ACS on STN
1979:434631 Document No. 91:34631 Biosynthesis of heparin. VI. Assay and properties of the microsomal uronosyl C-5 **epimerase**. Jacobsson, Ingvar; Baeckstroem, Gudrun; Hoeoek, Magnus; **Lindahl, Ulf**; Feingold, David S.; Malmstroem, Anders; Roden, Lennart (Biomed. Cent., Swed. Agric. Univ., Uppsala, S-751 23, Swed.). Journal of Biological Chemistry, 254(8), 2975-82 (English) 1979. CODEN: JBCHA3. ISSN: 0021-9258.

AB The formation of L-iduronic acid residues by C-5 epimerization of D-glucuronic acid units at the polymer level during the synthesis of heparin involves

exchange of the H atom at C-5 with protons of the medium (Lindahl, U., et al, 1976). When a heparin precursor polysaccharide composed of alternating D-glucuronosyl-5-3H and N-sulfated D-glucosaminosyl residues was incubated with a microsomal mouse mastocytoma preparation, the 3H located on C-5 was exchanged with protons of the medium. The rate of 3H release was directly proportional to the concentration of microsomal enzyme as well as to substrate concentration and was used as an assay for the uronosyl 5-**epimerase**. The reaction had a pH optimum close to 7.4. The reaction rate was strongly dependent on the ionic strength of the medium. Incubation of various 5-3H-labeled heparin precursor polysaccharides showed that only N-sulfated prepns. were substrates; the best substrate consisted largely of alternating D-glucuronosyl and N-sulfated D-glucosaminosyl moieties. Exhaustive incubation of this substrate with microsomal enzyme caused release of 60-70% of the 3H originally present; concomitantly, the L-iduronic acid content increased from 18 to 29% of the total uronic acid. When the reaction mixture was supplemented with 3'-phosphoadenylylsulfate, >50% of the substrate was converted into an O-sulfated species which contained 41% L-iduronic acid. Thus, although exchange of the C-5 H atom always accompanies 5-epimerization, the converse may not always occur. In contrast, the membrane-bound endogenous intermediates did not lose 3H in excess of the extent of conversion of D-glucuronic acid to L-iduronic acid. Apparently, the high degree of organization in the native biosynthetic system permits a stricter regulation such that each attack by the **epimerase** is carried through to conversion of configuration.